SUPPLEMENTARY INFORMATION

Supplementary Methods

Plasmids and shRNA sequences

A TEV protease cleavage site (ENLYFQG) followed by a FLAG tag was introduced via PCR into the APP695 sequence and subcloned in peak12-APP (Lichtenthaler et al., 2003) with PmlI/NotI to obtain peak12-APP-TEV-FLAG. peak12 vectors expressing APPsβ, APPsβ' or APPsα ending in amino acids 14, 15 or 16 of the Aβ sequence were amplified via PCR and subcloned into PmlI/NotI digested peak12-APP vector. HindIII/NotI digested APP-TEV-FLAG was subcloned into HindIII/NotI digested pCR8/GW/TOPO-pCS2-MCS to obtain pCR8/GW/TOPO-APP-TEV-FLAG. FU-ΔZeo was generated by digestion of FU-Valentin vector (Lois et al., 2002) with BstZ17I/PMEI followed by religation. FU-ΔZeo-DsRed-UAS-GW was generated by subcloning the SnaBI/NheI digested DsRed-UAS-GW cassette of pT2D-Dest_pA_DsRed.T4_E1b_UAS_E1b_GW-R1-R2_pA provided by Christian Haass (Paquet et al., 2009) into AfeI/NheI digested FU-ΔZeo vector. FU-ΔZeo-Gal4-VP16 was generated by PCR amplification of Gal4-VP16 from pT2D-Exp_EF1a_Gal4VP16_pA provided by Christian Haass (Paquet et al., 2009). The PCR product was subcloned into NheI/NotI digested FU-ΔZeo vector. FU-ΔZeo-DsRed-UAS-APP-TEV-FLAG was generated by Gateway cloning L/R recombination between the donor plasmid pCR8-GW-TOPO-APP-TEV-FLAG and FU-ΔZeo-DsRed-UAS-GW. pLVTHM was described (Wiznerowicz and Trono, 2003). To obtain pLVTHMmod the H1 element was amplified by PCR and subcloned into pLVTHM vector with BamHI/XmaI. shRNA oligo duplexes targeting ADAM10 (AD10) were ligated into MluI/XmaI digested pLVTHMmod vector to obtain lentiviral knockdown plasmids plVTHMmod-Control,-sh6, -sh7, -sh9, -MM8. Sh9 sequence was adapted from the most potent siRNA of the Dharmacon pool. pLKO2mod-EGFP-WPRE was generated by replacing the hairpin of pLKO1-mmADAM10-TRC44 (RNAi consortium) with an MluI/XmaI site and a subsequent replacement of the puromycin resistance gene with EGFP-WPRE. shRNA oligo duplexes targeting murine (mmA10) and human AD10, murine AD9, murine AD17 and human BACE1 (B1) were ligated into MluI/XmaI digested pLKO2mod-EGFP-WPRE vector to obtain pLKO2mod-EGFP-WPRE-mmA10-sh1, mmA10-sh2, B1-sh1, B1-Sh2, mmA17-sh1, mmA9-sh1. pcDNA 3.1(-)-VSV-G was generated by digestion of phCMV-VSV-G with SalI/EagI which was subcloned into XhoI/EagI digested pcDNA3.1(-). psPAX2 was from Didier Trono (Addgene Plasmid 12260).

Supplementary References

Lichtenthaler, S.F., Dominguez, D.I., Westmeyer, G.G., Reiss, K., Haass, C., Saftig, P., De Strooper, B. and Seed, B. (2003) The cell adhesion protein P-selectin glycoprotein ligand-1 is a substrate for the aspartyl protease BACE1. *J Biol Chem*, **278**, 48713-48719.

Lois, C., Hong, E.J., Pease, S., Brown, E.J. and Baltimore, D. (2002) Germline transmission and tissue-specific expression of transgenes delivered by lentiviral vectors. *Science*, **295**, 868-872.

Paquet, D., Bhat, R., Sydow, A., Mandelkow, E.M., Berg, S., Hellberg, S., Falting, J., Distel, M., Koster, R.W., Schmid, B. and Haass, C. (2009) A zebrafish model of tauopathy allows in vivo imaging of neuronal cell death and drug evaluation. *J Clin Invest*, **119**, 1382-1395.

Wiznerowicz, M. and Trono, D. (2003) Conditional suppression of cellular genes: lentivirus vector-mediated drug-inducible RNA interference. *J Virol*, **77**, 8957-8961.

Supplementary Table 1: shRNA sequences

Name:	shRNA sequence
Con	5'-CCCCcaacaagatgaagagcaccaaTTCAAGAGAttggtgctcttcatcttgttg TTTTTGGAAA-3'
sh6	5'-CCCCaagttgcctcctcctaaaccactTTCAAGAGAgtggtttaggaggaggcaacttTTTTTGGAAA-3'
sh7	5'-CCCCgacatttcaacctacgaatttTTCAAGAGAaaattcgtaggttgaaatgtcTTTTTGGAAA-3'
sh9	5'-CCCCggacaaacttaacaacaatTTCAAGAGAattgttgttaagtttgtccTTTTTGGAAA-3'
B1-sh1	5'-CCCCggtacaaagactgcgtcttgattcaagagatcaagacgcagtctttgtaccTTTTTGGAAA-3'
B1-sh2	5'-CCCCgcgtgacagaacagagaaatcttcaagagagatttctctgttctgtcacgcTTTTTGGAAA-3'
A10-sh1	5'-CCGGtagacattatgaaggattatcctcgaggataatccttcataatgtctaTTTTTGGAAA-3'
A10-sh2	5'-CCGGcagacttggctctcgataaacctcgaggtttatcgagagccaagtctgTTTTTGGAAA-3'
shA9	5'-CCGGgctcctgcacctcctttatatctcgagatataaaggaggtgcaggagcTTTTTGGAAA-3'
shA17	5'CCCCgcagcactccataaggaaactcgagtttccttatggagtgctgcTTTTTGGAAA-3'

Sequences sh6, sh7 and sh9 are against human ADAM10, A10-sh1 and A10-sh2 against murine ADAM10. shA9 is against murine ADAM9, shA17 against murine ADAM17 and B1-sh1 and B1-sh2 are against human BACE1. Sh7 has been described previously (Reiss et al., 2005).

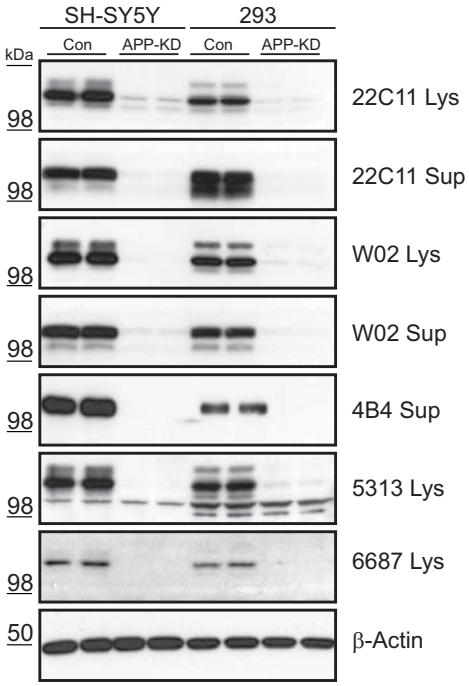
Supplementary Table 2: Primer sequences for quantitative RT-PCR

Genes	Primer sequences	Product size (bp)
ADAM9	For 5'-GTTTACACCTACGACAAGGAAGGC-3' Rev 5'-TCCCTCCACATAGCCTCGATAGT-3'	87
ADAM10	For 5'-TAAGGAATTATGCCATGTTTGCTGC-3' Rev 5'-ACTGAACTGCTTGCTCCACTGCA-3'	91
ADAM17	For 5'-TTGGAGCAGAACATGACCCTGATGG-3' Rev 5'-TGCAGCAGGTGTCGTTGTTCAGGTA-3'	276
Actb	For 5'-ATGCTCCCCGGGCTGTAT-3' Rev 5'-CATAGGAGTCCTTCTGACCCATTC-3'	87
GapDH	For 5'-TGTGTCCGTCGTGGATCTGA-3' Rev 5'-TTGCTGTTGAAGTCGCAGGAG-3'	150
Tbp	For 5'-GGCCTCTCAGAAGCATCACTA-3' Rev 5'-GCCAAGCCCTGAGCATAA-3'	107

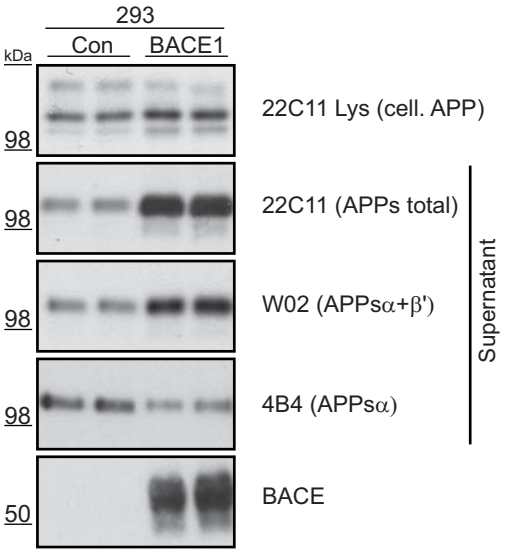
Suppl. Fig. S1: Specificity of APP antibodies. HEK293 and SH-SY5Y cells expressing endogenous APP were transfected with a control siRNA pool (Con) or a siRNA pool against APP (APP-KD). Cell lysates (Lys) were analyzed for APP with 22C11, 6687, 5313 and W02. Conditioned media (Sup) were analyzed for APP with 22C11, W02 and 4B4. As a loading control, cell lysates were probed for β-actin. APP was efficiently knocked-down. The blots show the specificity of the antibodies for APP.

Suppl. Fig. S2: Overexpression of the β-secretase BACE1 reduces 4B4 signal. Conditioned media and cell lysates of HEK293 cells either transfected with an empty control vector or BACE1 were analyzed for endogenous cellular APP (22C11), α+β'-cleaved APP (WO2), α-cleaved APP (4B4) and BACE1 as transfection control. BACE1 transfection reduced α-secretase cleavage and 4B4 signal, as expected.

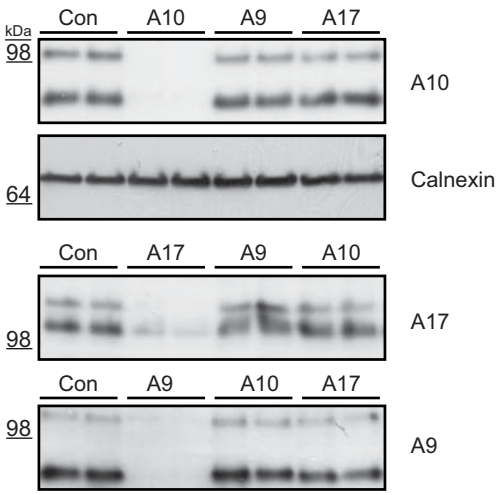
Suppl. Fig. S3: Knockdown of either ADAM9, 10 or 17 does not alter the protein levels of immature and mature forms of the other proteases. Membrane preparations of SH-SY5Y cells either treated with siRNA pools against ADAM9, 10, 17 (A9, A10, A17) or a control siRNA pool (Con) were probed for ADAM9, 10 and 17. As loading control membranes were analyzed for calnexin.


SH-SY5Y
293
Con
APP-KD
Con
APP-KD
kDa
98
22C11 Lys
98
22C11 Sup
98
W02 Lys
98
W02 Sup
98
4B4 Sup
98
5313 Lys
98
6687 Lys
50
β-Actin

Suppl. Fig.S1


293
Con
BACE1
kDa
98
98
98
98
50
22C11 Lys (cell. APP)
22C11 (APPs total)
W02 (APPsα+β')
4B4 (APPsα)
BACE
Supernatant

Suppl. Fig. S2


Con
A10
A9
A17
kDa
98
A10
64
Calnexin
Con
A17
A9
A10
98
A17
Con
A9
A10
A17
98
A9

Suppl. Fig.S3